Research paper

Synergistic, additive and antagonistic impacts of drought and herbivory on *Pinus sylvestris*: leaf, tissue and whole-plant responses and recovery

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Forests typically experience a mix of anthropogenic, natural and climate-induced stressors of different intensities, creating a mosaic of stressor combinations across the landscape. When multiple stressors co-occur, their combined impact on plant growth is often greater than expected based on single-factor studies (i.e., synergistic), potentially causing catastrophic dysfunction of physiological processes from an otherwise recoverable situation. Drought and herbivory are two stressors that commonly co-occur in forested ecosystems, and have the potential to 'overlap' in their impacts on various plant traits and processes. However, the combined impacts from these two stressors may not be predictable based on additive models from single-stressor studies. Moreover, the impacts and subsequent recovery may be strongly influenced by the relative intensities of each stressor. Here, we applied drought stress and simulated bark-feeding herbivory at three levels of intensity (control, moderate and severe) in a full factorial design on young *Pinus sylvestris* L. seedlings. We assessed if the combined effects from two stressors were additive (responses were equal to the sum of the single-factor effects), synergistic (greater than expected) or antagonistic (less than expected) on a suite of morphological and physiological traits at the leaf-, tissue- and whole-plant level. We additionally investigated whether recovery from herbivory was dependent on relief from drought. The two stressors had synergistic impacts on specific leaf area and water-use efficiency, additive effects on height and root-to-shoot ratios, but antagonistic effects on photosynthesis, conductance and, most notably, on root, shoot and whole-plant biomass. Nevertheless, the magnitude and direction of the combined impacts were often dependent on the relative intensities of each stressor, leading to many additive or synergistic responses from specific stressor combinations. Also, seedling recovery was far more dependent on the previous year's drought compared with the previous year's herbivory, demonstrating the influence of one stressor over another during recovery. Our study reveals for the first time, the importance of not only the presence or absence of drought and herbivory stressors, but also shows that their relative intensities are critical in determining the direction and magnitude of their impacts on establishing seedlings.

Keywords: boreal forest, disturbance, photosynthesis, pine weevil, Scots pine, seedlings, stressors.

Introduction

Under natural conditions, the realized productivity of plants is often below potential productivity owing to the influence(s) of one or more external stressors (i.e., disturbance and stress; Grime 1977). A disturbance is a single or episodic event that disrupts the current growth conditions and results in total or partial destruction of plant biomass, while stress is a sustained deviation from optimal environmental conditions that causes reduced growth rates (Pickett and White 1985). When

disturbances and stress occur simultaneously, their interactive effects on plant performance are often synergistic (greater than expected based on single-factor studies) (Niinemets 2010), resulting in a catastrophic disruption of functional processes (Sih et al. 2004). Under natural conditions, overlapping of multiple environmental stressors are the rule rather than the exception (Chapin et al. 1987, Vierling and Kimpel 1992). Moreover, most ecosystems experience additional anthropogenic disturbances that also interact with natural stressors (Raffa et al. 2008). The ecological consequences of multiple stressors are difficult (or impossible) to predict based on studies in which only individual factors were varied (Lotze and Worm 2002, Collins and Smith 2006), and are dependent on the duration, frequencies and intensities of each individual stressor (Christensen et al. 2006, Desprez-Loustau et al. 2006, Rennenberg et al. 2006), as well as the tolerance and recovery potential of the species within the system (Brodribb and Cochard 2009, Vinebrooke et al. 2004). Given that more severe, frequent and novel combinations of stressors are expected with global change (Vinebrooke et al. 2004, Williams and Jackson 2007), understanding their interactive effects is crucial (but poorly investigated) in predicting how plants will perform under these changed conditions.

Boreal forests cover c. 29% of global forest area, sequester and store vast amounts of global carbon (C), regulate global climate, provide timber resources and are home to a diverse assemblage of plants and animals (Anderson 1991, Bonan et al. 1992). These high-latitude forests are subjected to several natural and anthropogenic stressors that may synergistically interact to reinforce their individual impacts. Disturbances from herbivorous insects are among the most common natural stressors in boreal forests (Schowalter et al. 1986, Ayres and Lombardero 2000). Bark-feeding insects, such as the large pine weevil (Hylobius abietis L.), have caused devastating impacts on forest regeneration due to their preference for mass propagating on clear-cut coniferous sites and feeding on establishing seedlings (Leather et al. 1999, Scott and King 1974). Mechanical scarification of the soil is a common anthropogenic disturbance intended to reduce stress from herbivorous insects and competing vegetation for establishing tree seedlings (Sutton 1993, Björklund et al. 2003, Örlander and Nordlander 2003). However, the surface soil after scarification retains less water than undisturbed soils (Nilsson and Örlander 1995), which can impose water stress on seedlings, and may increase their susceptibility to attack by any remaining herbivores (Mattson and Haack 1987, Selander and Immonen 1992, Dobbertin et al. 2007). Moreover, the frequencies of dry years, the duration of dry periods and incidence rate of insect outbreaks are expected to increase in the boreal forest with climate change (Ayres and Lombardero 2000, Carroll et al. 2004, Schär et al. 2004), and therefore we can expect an increase in the co-occurrence and intensity of herbivory and drought stressors, particularly in forests that have been artificially disturbed to stimulate regeneration.

While drought and herbivory have each been studied individually, no research that we know of has assessed the combined effects of these common stressors on boreal tree seedlings. The present study was designed to investigate the effects of these two stressors individually and in combination on *Pinus sylvestris* L. seedlings in a simulated, anthropogenically disturbed boreal forest environment (e.g., clear-cut with scarified soils). We applied three levels of each stressor (control, moderate and severe) to all seedlings during their first growing season. During the second season, we alleviated herbivory for all seedlings, but maintained drought stress for half of the seedlings. Our primary hypothesis was that drought stress and herbivory would both adversely affect morphological and physiological plant traits at the leaf-, tissue- and whole-plant level, and that the combined effect from both stressors would be synergistic. Alternatively, the effects of one stressor would overwhelm the effects of the other and thus the combined effect would be less than expected from an additive response (e.g., an antagonistic effect). We also hypothesized that seedlings would recover from drought and herbivory when one or both stressors were alleviated, but that the extent of recovery would be constrained for seedlings that experienced severe (compared with moderate) drought during the previous year, and recovery from one stress (herbivory) would be less for seedlings that were exposed to a second year of drought stress. In doing this research, we will reveal the underlying mechanisms of how two of the most common and detrimental stressors in boreal forests impact the performance of establishing seedlings under anthropogenically disturbed conditions.

Materials and methods

Study site, species and growing media

An experiment was set up in June 2009 at the Faculty of Forest Sciences in Umeå, Sweden (63°50′N, 20°15′E) to simulate the growing conditions for tree seedlings in a boreal forest clear-cut. Two hundred and sixteen mesocosms were constructed using 10-liter plastic pots (222 mm in height and 280 mm in diameter) with a plastic net lining (1.5 mm mesh size) that fit closely to the bottom and walls of the pot interior. The mesh lining provided enough structural support to hold the soil intact without the presence of the plastic pots, and allowed the soil profile to exchange air/water with the surrounding environment. Each mesocosm was filled with a 30 mm base layer of industrial quartz-sand, upon which a 30 mm layer of organic material and another 125 mm of mineral soil were placed. The resulting sequence of organic material and mineral soil was intended to resemble the planting spots created by mounding site preparation (cf. Sutton 1993).

The organic layer and top mineral soils were collected from mixed conifer forests near Umeå, Sweden in May 2009. Intact 1 × 1 m sections of moss carpets and underlying humus were processed in a heavy-duty compost-mincer and then passed through a sieve (25 mm mesh) to produce a bulk sample of coarse organic material. The mineral soil was a fine-textured glacial till that was passed through a sieve (4 mm mesh) to remove stones and roots.

On 19 June 2009, after 1 week of acclimation to outdoor conditions, we planted a 1-year-old containerized *P. sylvestris* seedling in the center of each mesocosm. With a planting depth of ~100 mm, the initial root systems were situated just above the organic layer of the soil profile. The initial mean root collar diameter, shoot height, total dry weight and root-to-shoot ratio of seedlings were 1.5 ± 0.03 mm, 51.0 ± 1.4 mm, 0.48 ± 0.02 g and 0.55 ± 0.02, respectively. The seedlings originated from Västerhus seed orchard seeds and were delivered from freeze storage in Kilåmon, Sweden Skogsplantor on 12 June 2009.

Treatment applications and experimental design

At the time of planting, three drought treatments were applied to the mesocosms by trimming the plastic pot to three different heights: (i) 222 mm (no trimming—control); (ii) 105 mm (moderate drought); or (iii) 55 mm (severe drought). The trimming exposed the net lining so that the effective surface area for evaporation increased by 2.1 and 2.7 times for the moderate and severe drought treatments, respectively. An increase in evaporative surface area is a common occurrence under field conditions following typical mounding site preparation (Sutton 1993). The trimming resulted in a gradient of soil volumetric water content (VWC) across the treatments (see Figure S1 available as Supplementary Data at *Tree Physiology* Online). The average VWC of the top 50 mm soil ranged from 3 to 30% across all treatments during the course of the study (June 2009–December 2010); the average VWC of the moderate and severe drought mesocosms were 70 and 58% that of the control, respectively, based on repeated manual measurements throughout the study period. The wilting point of these soils corresponded to a VWC of 5–11% (Magnusson 1992), which occurred after 3–5 days without rainfall in the moderate and severe drought mesocosms and after 6–7 days in the control mesocosms.

In order to simulate herbivory from the large pine weevil, we applied three levels of feeding activity: (i) no scars (no herbivory—control); (ii) one scar (moderate herbivory); or (iii) two scars (severe herbivory) at the seedling stem base. The scars were applied by using a scalpel to scrape off a patch of stem bark and phloem that was ~10 mm in vertical length and each covered approximately one-third of the circumference of the stem. When two scars were applied, they were positioned closely above each other with one scar moved laterally to disrupt about two-thirds of the phloem along the stem. The scarring was done twice on seedlings: at the time of planting (June) and then again at the end of the first growing season (September). The second scarring was applied to a previously unharmed section of the stem. These applications mimic the natural feeding behavior of the large pine weevil over a growing season (Day et al. 2004).

The experiment was set up in a fully randomized complete block design arranged in 24 blocks. Each block contained nine treatment combinations with all possible pair-wise combinations of the three levels of drought intensity and the three levels of herbivory. The blocks were oriented in a north to south direction and placed on a sand bed covered by fiberglass-cloth in an outdoor exclosure. The climatic conditions during the study period fell within the normal pattern except for a high July precipitation in 2009 (see Figure S2 available as Supplementary Data at *Tree Physiology* Online). Natural precipitation was the primary water supply to the seedlings, with some additional light watering prior to planting and after dry spells as a precaution to avoid lethal desiccation. The mesocosms were monitored regularly (see Methods 1 available as Supplementary Data at *Tree Physiology* Online) until harvest, 19 months after initiation.

In order to assess recovery from herbivory under droughted and non-droughted conditions, no scarring was applied during the second year and 12 (half) of the blocks were randomly selected to receive relief from the drought treatments (while the other half had sustained drought). Relief from drought was applied on 18 May 2010 by restoring the outer plastic of the mesocosm, so that each mesocosm obtained a full-height of 222 mm and VWCs equivalent to the control pots. The other 12 blocks had the drought treatments sustained.

Morphological traits

Stem diameters at the base of the stem, shoot lengths and needle lengths were measured on each seedling at outplanting, and at the end of the first and second growing season. Radial growth was calculated as the increase in stem diameter from the time of outplanting to the end of the first season, and then again from the end of the first to the end of the second growing season. At harvest during December 2010, the current (C) and the 1-year-old (C + 1) needles and stem axes of each seedling were separated from the initial shoot tissues and the dry weights of all fractions were determined individually. The roots were separated from soil by gently washing the root system in water. Ten fresh needles from C and C + 1 fractions of each seedling were laid flat (with a reference scale) and digitally photographed. Projected areas and needle lengths were quantified using image processing software (Image J, Scion Co., Fredrick, MD, USA). All plant material was then dried at 60 °C for at least 48 h and weighed (±0.1 mg). These quantified areas and masses were used to calculate

specific leaf areas (SLAs) by dividing the leaf area by leaf mass.

Physiological traits

We measured light-saturated instantaneous net photosynthesis (A, μmol CO_2 m^{-2} s^{-1}), stomatal conductance to water vapor (g_s, mol H_2O m^{-2} s^{-1}) and water-use efficiency (WUE; A g_s^{-1}) using a portable photosynthesis instrument equipped with a CO_2 controller and an attached light source (LI-6400, Li-Cor Biosciences, Inc., Lincoln, NE, USA). We used an opaque conifer chamber (model LI-6400-18) that was large enough to enclose up to 6 cm of the current apical shoot. Chamber conditions were set to 1500 ppfd, 400 ppm CO_2, 20 °C and 60–80% relative humidity. Measurements were made in September 2009 and July 2010. We used 8 blocks of seedlings ($n = 9$ seedlings per block) in the first year and 23 blocks in the second year for gas exchange measurements. Following the measurements, we photographed the silhouette of the measured section of each shoot to normalize gas exchange rates on an area basis.

Data analysis

For data from the first growing season, two-way analysis of variance (ANOVA) was used to test for the main effects of drought and herbivory and their interaction on all response variables across all treatments. For the second year, a three-way ANOVA was used to test for the effects of drought, herbivory, relief and all interactions. The herbivory treatments were not applied during the second year, thus in the three-way ANOVA a significant effect from herbivory indicates incomplete recovery from the previous year's herbivory (i.e., a legacy effect), while a significant herbivory × relief interaction indicates that recovery from herbivory was dependent on relief from drought. Block and its interactions with drought and herbivory were random factors in each test. Tukey's honestly significant difference tests were used to compare the means of drought and herbivory treatments within each year. For ANOVAs, when necessary variables were natural log-transformed to meet the assumptions of normality and homoscedasticity of error variance. All pairwise comparisons were made on untransformed data.

To determine whether drought and herbivory treatments exerted additive, synergistic or antagonistic impacts on *P. sylvestris* trait, we compared the observed (Obs) effects with the expected (Exp) additive effects for the seedlings exposed to both stressors (see Methods 2 available as Supplementary Data at *Tree Physiology* Online for details of calculations). Expected additive effects from two stressors were calculated based on the sum of effect sizes from single stressors, and were then compared with the actual Obs from two stressors. When the difference between Obs and Exp was positive (i.e., Obs − Exp > 0) and the lower 95% confidence limit was

greater than zero, the impact from the combined stressors was classified as synergistic. Similarly, when the difference between Obs and Exp from combined stressors was negative (i.e., Obs − Exp < 0) and the upper 95% confidence limit was less than zero, the combined impact was classified as antagonistic. When the 95% confidence interval (95% CI) crossed the zero line, the impact was considered additive. For the first growing season, we used seedlings from all the measured blocks, and for the second season we based our calculation on the seedlings with sustained drought.

Results

Morphological traits

Out of the eight morphological response traits we measured during the first year, drought had negative effects on seedling shoot mass (Figure 1a, left subpanel; $P < 0.001$) and stem mass ($P < 0.001$) and herbivory had negative effects on needle mass ($P = 0.02$) and needle area ($P < 0.001$). Values of SLA (Figure 1b, left subpanels) were affected by both drought ($P < 0.001$) and herbivory ($P < 0.001$) treatments, but not their interaction. Shoot and needle lengths (Figure 1c and d) were not affected by either stressor or their interaction during the first year of the study (all $P > 0.1$). Herbivory had a positive effect on radial growth (Figure 1e; $P < 0.001$). During the second year, all morphological traits were negatively influenced by sustained drought treatments (Figure 1, right subpanels; all $P < 0.001$), while only values of SLA (Figure 1b, right subpanel) were affected by herbivory ($P < 0.001$), and the interaction of herbivory with drought ($P = 0.04$). Herbivory had a stronger impact on needle area than needle mass ($P < 0.001$), which contributed to the observed changes in SLA. There were also strong drought × relief interactions on all the morphological parameters (all $P < 0.01$) except the shoot length, which appeared due to a stronger effect of the drought treatments in the case when drought was sustained compared with the case when drought was relieved (Figure 1, right subpanels).

Physiological traits

For gas exchange parameters during the first year (Figure 2, left subpanels), photosynthesis was negatively affected by herbivory ($P < 0.01$), conductance was negatively affected by both drought ($P = 0.05$) and herbivory ($P < 0.01$) and WUE was affected by drought ($P < 0.001$), herbivory ($P = 0.05$) and their interaction ($P = 0.04$). The interactive effect of drought and herbivory on WUE appeared due to the positive effect of herbivory on WUE only for seedlings given the moderate drought treatment (Figure 2c, left subpanel). During the second year (Figure 2, right subpanels), photosynthesis, conductance and WUE were all affected by drought (all $P < 0.001$), but not by herbivory. Photosynthesis, conductance and WUE all
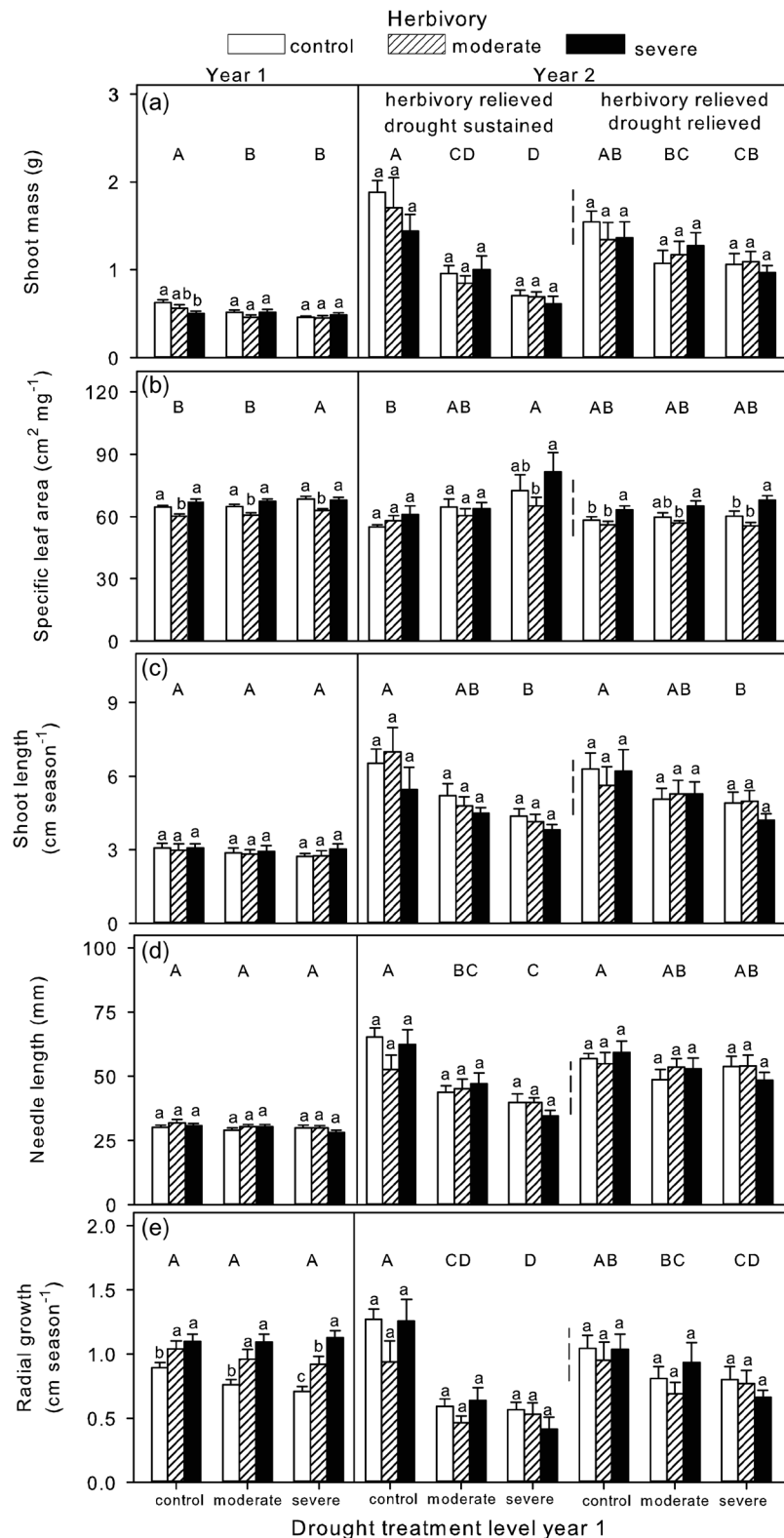
Figure 1. Drought and herbivory are two common environmental stressors that impact seedling growth and morphology. To test the effect of multiple stressors on tree seedling morphological traits, seedlings of *P. sylvestris* were subjected to three levels of drought (control, moderate and severe) and three levels of herbivory (control, moderate and severe) in a full factorial design during Year 1 of the study (left subpanels), and then in Year 2, all the seedlings were given relief from herbivory and half the seedlings had the drought treatments sustained and half were given relief from drought stress (right subpanels). Seedling response traits (means ± 1 SE) include shoot mass (a), specific leaf area (b), shoot length (c), needle length (d) and radial growth (e). Within each group of three bars, different lower case letters indicate significant differences between herbivory treatments, and within each year different capitalized letters indicate significant differences between drought treatments.
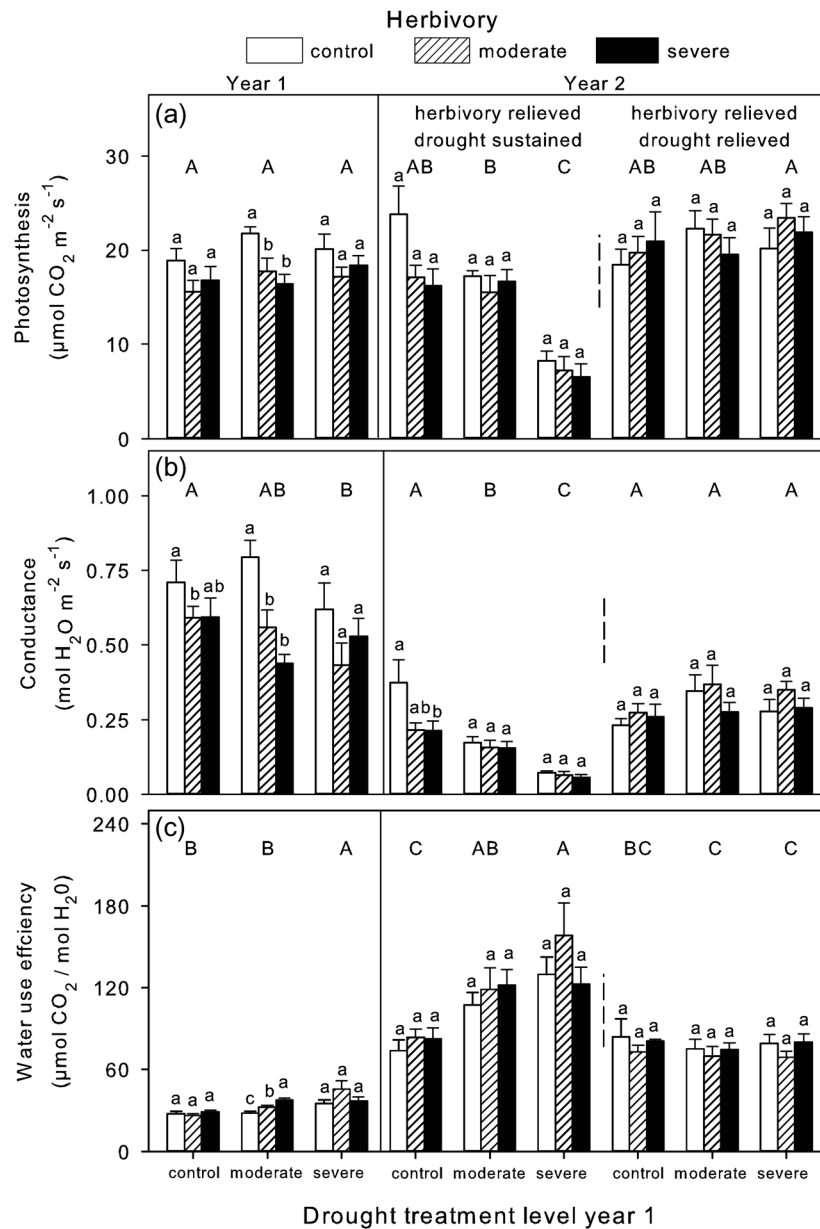
Figure 2. Drought and herbivory are two common environmental stressors that impact seedling photosynthetic and conductance rates. To test the effect of multiple stressors on tree seedling gas exchange rates, seedlings of *P. sylvestris* were subjected to three levels of drought (control, moderate and severe) and three levels of herbivory (control, moderate and severe) in a full factorial design during Year 1 of the study (left subpanels), and then in Year 2, all the seedlings were given relief from herbivory and half the seedlings had the drought treatments sustained and half were given relief from drought stress (right subpanels). Traits (means ± 1 SE) include photosynthesis (a), conductance (b) and water use efficiency (c). Within each group of three bars, different lower case letters indicate significant differences between herbivory treatments, and within each year different capitalized letters indicate significant differences between drought treatments.

had significant recovery following relief from drought stress in Year 2 compared with seedlings with sustained drought (all $P < 0.001$). Similar to morphological traits, there were also strong drought × relief interactions on gas exchange rates (all $P < 0.001$), which appeared due to a stronger effect of the drought treatments in the case when drought was sustained compared with the case when drought was relieved (Figure 2, right subpanels).

Final harvest

The cumulative effects of 2 years of treatments on the final morphological traits (Figure 3) were most evident as strong negative effects from drought, particularly when severe drought was sustained for 2 years (all $P < 0.001$). The main effects of the herbivory were apparent as a positive effect on stem diameter (Figure 3d; $P < 0.001$). Root-to-shoot ratios (Figure 3f) were positively affected by the drought treatment,
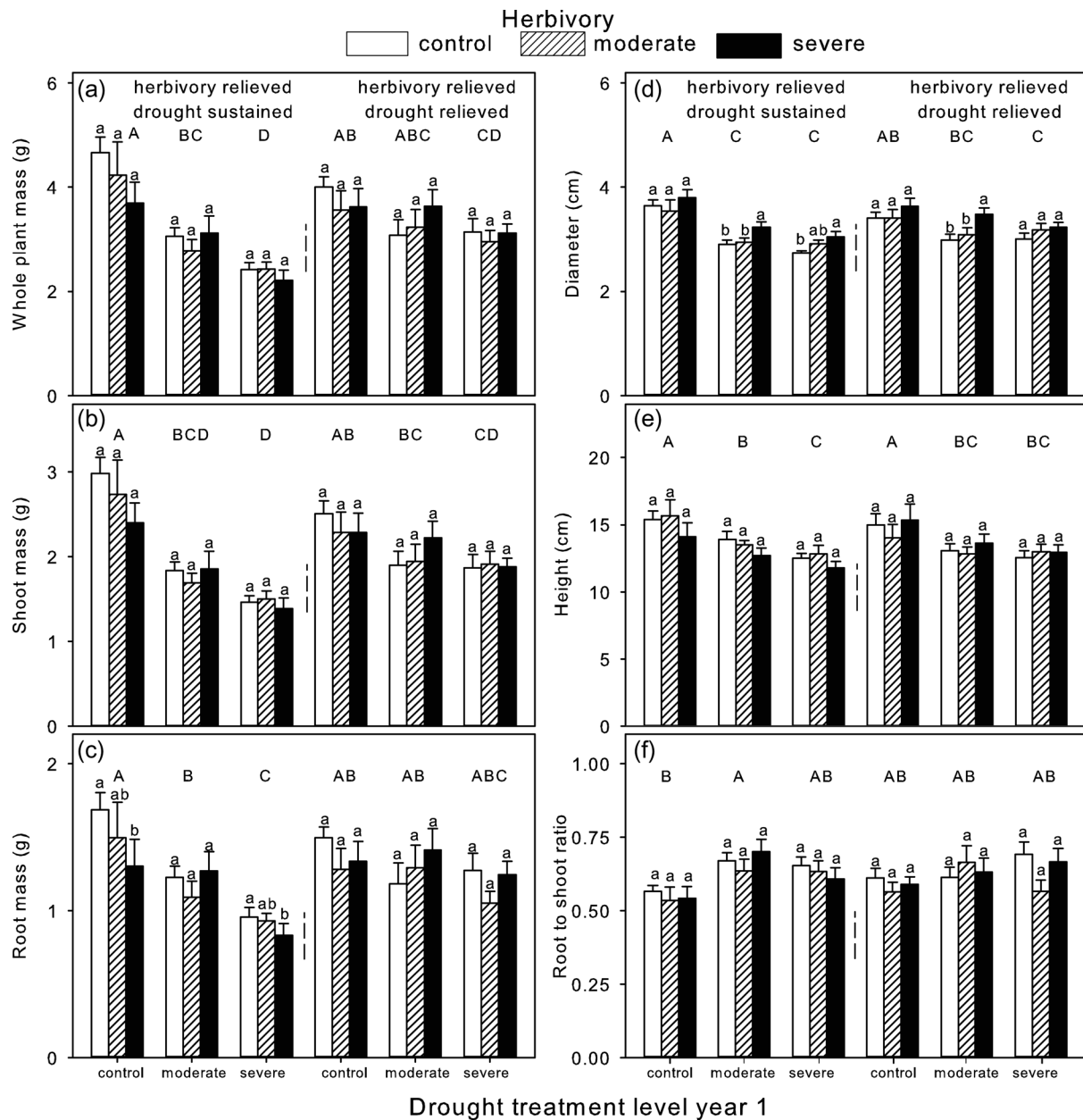
Figure 3. Drought and herbivory are two common environmental stressors that impact seedling growth and morphology. To test the cumulative 2-year effect of multiple stressors on tree seedling biomass and morphology at final harvest, seedlings of *P. sylvestris* were subjected to three levels of drought (control, moderate and severe) and three levels of herbivory (control, moderate and severe) in a full factorial design during Year 1 of the study and then in Year 2, all the seedlings were given relief from herbivory and half the seedlings had the drought treatments sustained and half were given relief from drought stress. Traits (means ± 1 SE) include whole-plant mass (a), shoot mass (b), root mass (c), stem diameter (d), height (e) and root-to-shoot ratio (f). Within each group of three bars, different lower case letters indicate significant differences between herbivory treatments, and within each year different capitalized letters indicate significant differences between drought treatments.

but there were no significant impacts from relief or the interaction of drought × relief. There was no overall significant effect from relief, but whole-plant mass, shoot mass, root mass and diameter had drought × relief interactions (all $P < 0.02$).

Synergistic, additive and antagonistic effects

Drought and herbivory in combination over the 2 years of the study generally had antagonistic effects on most of the morphological and physiological traits that we measured. This was particularly so for biomass and gas exchange parameters (Figure 4). Synergistic effects were found for both WUE and SLA (during the second year), and additive effects occurred for shoot height and root-to-shoot ratios (Figure 4). When the intensity of drought and herbivory was considered separately for each year, their direction and magnitude were often dependent on the specific combination of stressors (Figure 5). During the first season,
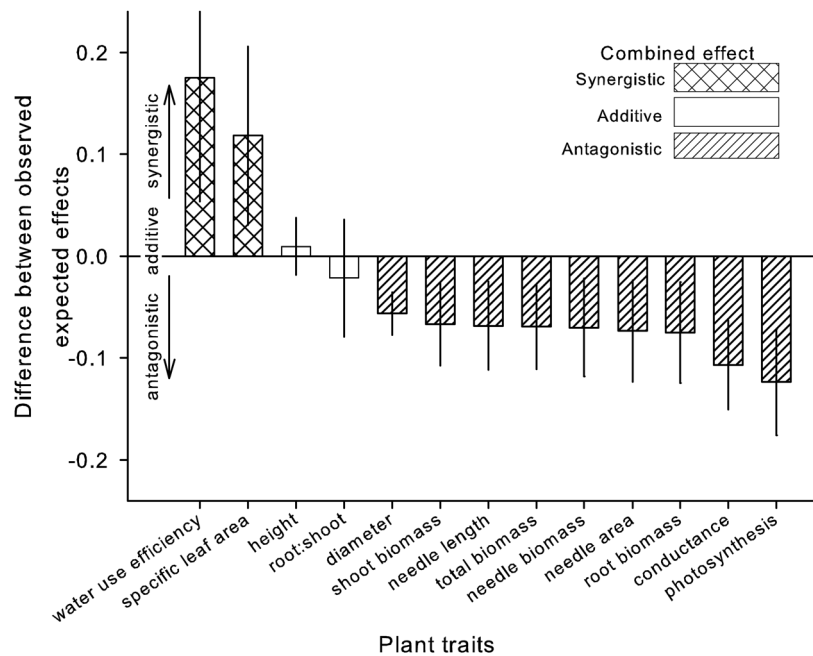
Figure 4. The combined impacts from drought and herbivory on various plant traits were synergist, additive or antagonistic (greater than, equal to or less than expected effects, respectively, based on single stressor effect sizes). The bars represent the overall effect size difference (mean ± 95% CI) between the observed and expected additive effects from combined drought and herbivory on morphological traits at final harvest and second-year physiological traits of *P. sylvestris* seedlings. The zero line represents the expected additive effects from combined stressors. When the means (and their 95% confidence limits) were greater than or less than the zero line they were considered synergistic or antagonistic, respectively. Trait data are from seedlings that received sustained drought for 2 years.

the combined stressors shifted from having additive to antagonistic effects on shoot, needle and stem biomass and radial growth with increasing combined stressor intensity (Figure 5a and b), while the effects were synergistic for needle length, but only when drought was severe (Figure 5c). During the second year, drought and herbivory had antagonistic effects on photosynthesis and conductance rates when drought was moderate, but were additive when drought was severe, independent of herbivory (Figure 5d and e). Synergistic effects during the second season were mainly found for WUE and SLA at both moderate and severe herbivory, but these effects were apparent only under specific drought combinations (Figure 5f and g). Synergistic effects were found for seedling height when both stressors were of moderate intensity (Figure 5h). Weak additive effects were generally found for total, shoot and root biomass at final harvest (at the end of Year 2) (Figure 5i–k), except when moderate drought and severe herbivory treatments were combined, which resulted in antagonistic effects. Drought and herbivory also operated in an additive manner on the ratio of root to shoot biomass for all treatment combinations (Figure 5l).

Discussion

Single stressors

Stress from drought and herbivory have the potential to cause serious disruptions to many physiological processes of establishing seedlings (Desprez-Loustau et al. 2006). Our direct comparison of these two stressors revealed that each stressor targeted a suite of plant traits related to plant C balance, albeit the magnitude of impact from drought was generally greater. Over 2 years of sustained drought, we observed a >50% reduction in photosynthetic area, >20% lower photosynthetic rates and 20–40% less root biomass. These multi-trait impacts on components of plant C and water balance provide a mechanistic demonstration of how environmental stressors such as drought can have broad-spectrum and long-term influences on plant performance (Barber et al. 2000, Galiano et al. 2011). Similarly, bark-feeding insects have the potential to impede tree seedling C balance and growth, although the mechanisms may differ from drought (i.e., consumption of C assimilates during phloem transport). One potential impact of both bark-feeding and drought is feedback inhibition of photosynthesis, caused by disruption of C transport (Krapp and Stitt 1995, Sala et al. 2010, McDowell 2011). Indeed, we found that herbivory (but not drought) had a strong negative impact on photosynthetic rates (and needle area and mass) during the year of active feeding. Further, we observed resin flow out of the weevil scars and compensatory re-growth around the wound for healing, which could additionally deplete large quantities of C reserves (Gref and Ericsson 1985, Baier et al. 2002, Gaylord et al. 2007). However, the scarring we applied and associated impacts on traits associated with C
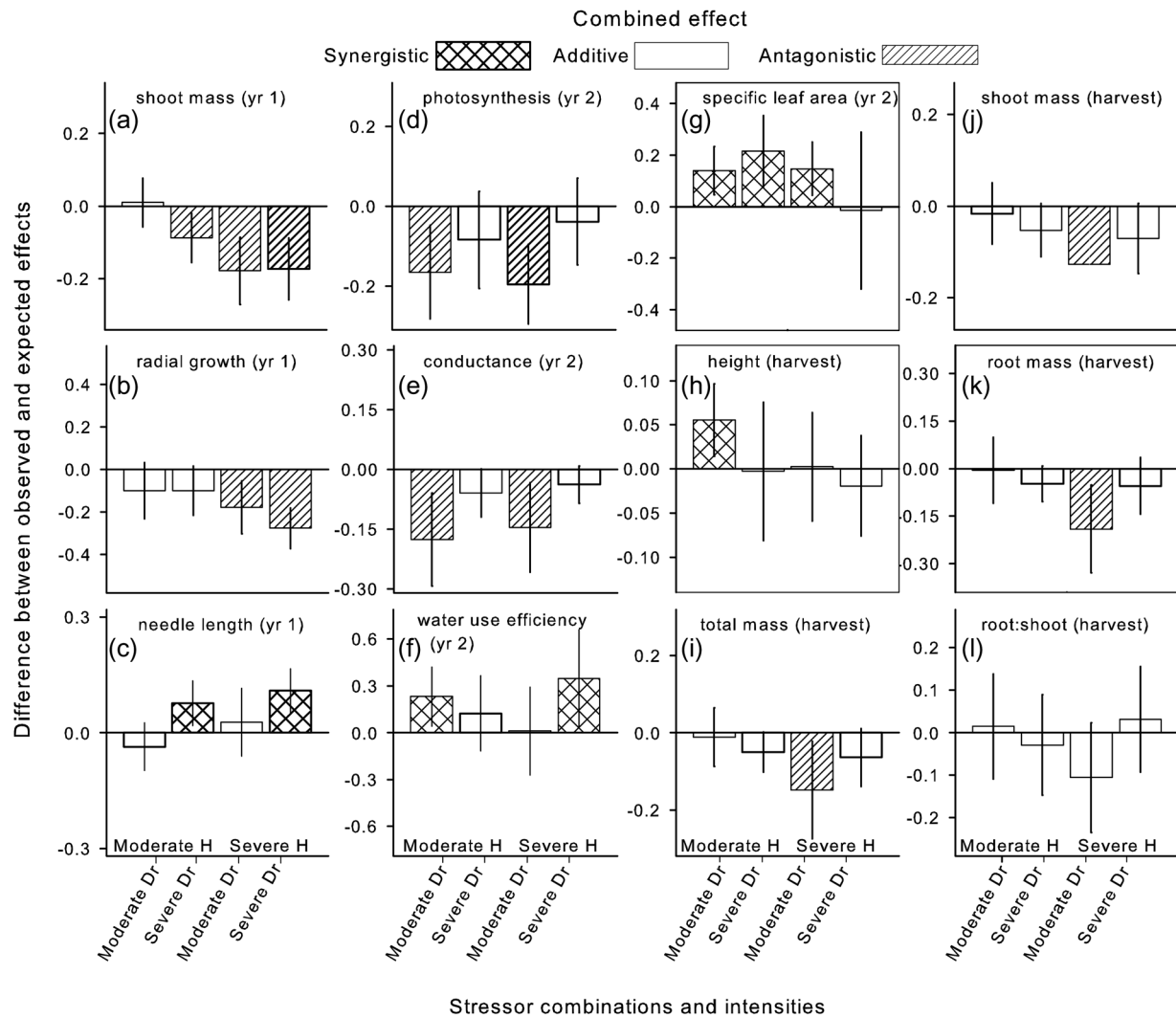
Figure 5. The combined impact from drought and herbivory on various plant traits were often dependent on the relative intensities of each stressor, leading to many synergistic, additive or antagonistic (greater than, equal to or less than expected effects, respectively, based on single stressor effect sizes) effects. The bars represent the intensity-specific effect size difference (mean ± 95% CI) between the observed and expected additive effects from combined drought (Dr) and herbivory (H) stressors on morphological and physiological traits of *P. sylvestris* seedlings for each year of the study. The zero line represents the expected additive effects from combined stressors. When the means (and their 95% confidence limits) were greater than or less than the zero line they were considered synergistic or antagonistic, respectively. Seedling response traits include shoot mass (a), radial growth (b) and needle length (c) from the first year (Year 1) of the study and photosynthesis (d), conductance (e), WUE (f) and SLA (g) from the second year of the study (Year 2) and height (h), total mass (i), shoot mass (j), root mass (k) and root-to-shoot ratio (l) at the final harvest (harvest). Trait data from the second year and at final harvest are from seedlings that received sustained drought for 2 years.

balance had a limited effect on overall growth, and thus the overall impact from herbivory was less than that from drought.

Multiple stressors

Our primary hypothesis regarding multiple stressors was that the combined impact from two stressors on plant performance would be equal to or greater (i.e., additive or synergistic, respectively) than the individual impacts from a single stressor. However, our data generally supported the alternative hypothesis that multiple stressors had antagonistic effects (i.e., less effect than expected based on the effect of each stressor in isolation) on most traits. This was most evident for total, shoot

and root biomass, as well as for gas exchange rates, in which the expected reductions in performance from two stressors were not as severe as expected. Nevertheless, there were two key functional traits, SLA and WUE, that were affected by both stressors and exhibited relatively strong, synergistic effects from their combined impact. These two traits are particularly important to resource use efficiency, C gain and allocation and survival (Reich et al. 1997). The synergistic increases in SLA and WUE from the combined effects from two stressors may have stimulated an increase in short-term C gain and water conservation, respectively (Meyer 1998). Consequently, these two synergistic effects appeared to help the establishing

seedlings cope with the impacts from multiple stressors. Studies on the effects of drought have reported increases in SLA and WUE with drought stress (Sands and Rutter 1959, van den Driessche 1991, Thorne and Frank 2009), although studies on herbivory are less consistent, and have shown reductions in SLA (Meyer 1993), thus demonstrating how studies on individual stressors cannot be used to predict effects from multiple stressors.

Many forests typically experience a mix of anthropogenic, natural and climate-induced stressors of different intensities, creating a mosaic of stressor combinations across the landscape, which may be critical in understanding the local and overall impacts of these stressors on forest regeneration (Galiano et al. 2012). Although the overall effects we observed from drought and herbivory on seedling traits were generally antagonistic, a more detailed analysis revealed that the impacts were not always antagonistic, but instead were dependent on the relative intensities of each stressor. For example, during the first year of the study, the cumulative effects from the two stressors on shoot biomass and radial growth were antagonistic when either stressor was severe, but the combined effects were stronger (additive) when both stressors were of moderate intensity. This suggests that co-occurring stressors at lower intensity could have a disproportionate, negative impact on seedling growth. However, seedling height and root-to-shoot ratios responded additively across all treatments, while needle length responded synergistically when drought stress was severe, irrespective of herbivory intensity, thus demonstrating how the effects of multiple stressors are also trait dependent. Intensity-dependent effects from multiple stressors have rarely been explored, but our results clearly indicate the importance of considering the intensity of each stressor.

Despite finding some interesting and important synergies between herbivory and drought for specific combinations of stressor intensities, the majority of the effects were still antagonistic, particularly at the whole-plant level. There are a few possibilities to explain why we did not observe stronger synergistic effects between herbivory and drought. First, the impact of one stressor may have triggered a series of physiological responses that 'primed' or protected the seedlings from a co-occurring second stressor (Leshem and Kuiper 1996, Bowler and Fluhr 2000, Rennenberg et al. 2006). Crosstalk between plant responses to abiotic and biotic stressors is known to occur frequently and is mediated by shared biochemical responses (Fujita et al. 2006). Second, our herbivory treatments were simulated by manual scraping of the bark and phloem, which reproduced the mechanical injury to the host plant, whereas actual feeding by the large pine weevil may have stimulated additional physiological responses or transmitted pathogenic agents (Leather et al. 1999). There is evidence that the weevil acts as a vector of the pathogenic fungi (Lévieux

et al. 1994), which may increase the chances of mortality from an otherwise recoverable condition. Third, the impact of drought stress overshadowed the effects of weevil scarring. This has been observed under field conditions as well, in which unusually dry conditions during the previous year made pine weevil damage to establishing seedlings nearly negligible (Nilsson and Örlander 1995).

Recovery from single and multiple stressors

We hypothesized that seedlings would recover from drought and herbivory when one or both stressors were alleviated, but that the extent of recovery would be dependent on the intensities and combinations of stressors. Contrary to our expectations, seedlings that received the higher intensity drought during the first year had comparable recovery during the second year to those seedlings that initially experienced only moderate drought. Moreover, at the whole-plant level, there were minimal differences in growth between seedlings that were and were not relieved from the moderate intensity drought from the first year. The mechanisms involved in achieving complete, partial or limited recovery from a moderate stressor compared with a severe stressor are critical for understanding ecosystem resilience. First, there are a number of intrinsic physiological mechanisms to compensate for prior, suboptimal growing conditions and disturbances (Kozlowski and Pallardy 2002). For example, low-level water stress may induce short-term stomatal closure (Chaves et al. 2003, McDowell et al. 2008), while severe drought may activate signaling pathways and gene regulation to induce acclimation (Hare et al. 1999) and to allow rapid recovery of physiological competence after suitable moisture conditions had returned (Kozlowski and Pallardy 2002). Second, differences in recovery could be explained by greater carbohydrate accumulation in the severely (compared with moderately) droughted plants because growth is more sensitive than photosynthetic C assimilation to drought (Sala et al. 2012), thus providing additional substrate for growth in the following year when drought stress was relieved. Third, greater recovery from severe drought stress could have been a benign consequence of reduced growth from the previous year (e.g., seedlings had smaller shoots and higher root to shoot ratios). When drought stress was relieved during the second year, these severely droughted plants had a relatively high supply of water relative to their needs (i.e., a self-correcting mechanism; Kozlowski and Pallardy 2002), thus making the magnitude of recovery greater and more evident. Indeed, these intrinsic and extrinsic mechanisms may explain why gas exchange rates of seedlings that were relieved from drought were higher than those of the control seedlings that were never droughted.

Multiple stressors can interact to influence recovery (Yan et al. 2004, Soliveres et al. 2011, Valois et al. 2011, Zunzunegui et al. 2011), although this has rarely been studied for tree

seedlings. We allowed all of our seedlings to recover from herbivory, but continued to expose half of the seedlings to sustained drought stress with the expectation that these seedlings would not fully recover from herbivory. Contrary to our hypotheses, we found that recovery was far more dependent on the previous year's drought compared with the previous year's herbivory. Other studies on pine (e.g., *P. pinaster*) have shown a large degree of tolerance and recovery to pine weevil attacks, with growth losses limited to the first year of infestation, even if the attacks continue for a second year (Sampedro et al. 2009). These results again demonstrate how the impacts from multiple stressors (on recovery in this case) cannot be predicted based on studies in which only a single stressor was applied (Christensen et al. 2006, Valois et al. 2011).

Conclusions

In our study, we examined the combined impacts from a common biotic and abiotic stressor on establishing *P. sylvestris* seedlings, both of which are expected to increase in frequency and intensity in the future (Schär et al. 2004, IPCC 2007). We showed that the combined impact from these two stressors was generally antagonistic on most plant traits, although the combined impacts could also be additive or even synergistic depending on the relative intensities of each stressor. These findings have many implications. First, the intensity-dependent responses to multiple stressors not only reveal, for the first time, the importance of the presence or absence of multiple stressors, but also show that their relative intensities are critical in determining the direction and magnitude of their impacts. This implies that anthropogenic disturbances, such as soil scarification, that affect insect populations and soil moisture can have a substantial influence on how these co-occurring stressors interact and affect seedling establishment. Second, by measuring a suite of plant traits from the leaf to whole-plant level, we reveal many of the underlying mechanisms that explain non-additive effects on plant traits from two of the most frequently co-occurring stressors in boreal forests. Given that drought conditions, extreme heat events and insect outbreaks are expected to be more frequent with climate change (Easterling et al. 2000), disentangling these mechanisms is critical to predicting plant performance and community dynamics in the future. Finally, our study indicates that the combined impact of drought and herbivory would overall not lead to catastrophic dysfunction of tree seedling processes and thus suggests that forests may be relatively 'safe' from synergies among these stressors.

Supplementary data

Supplementary data for this article are available at *Tree Physiology* Online.

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